Active Bacteria at the ERSP FRC

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Problem

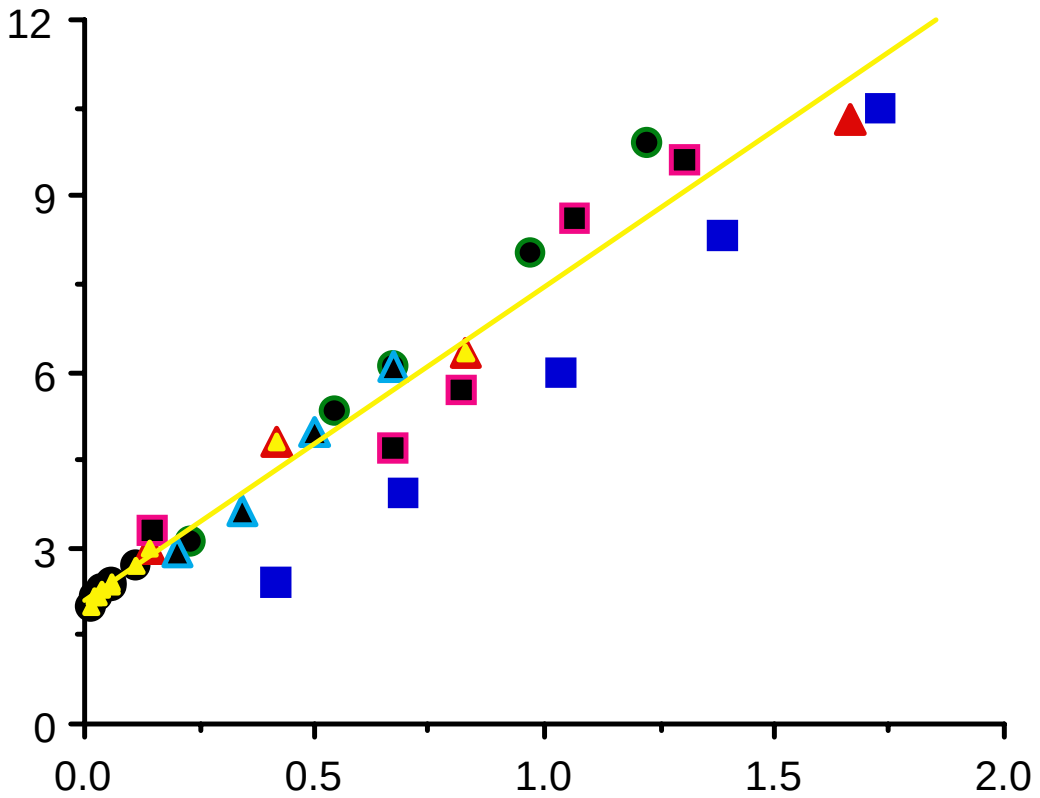
Molecular methods can to identify specific groups of microorganisms in complex samples in a culture independent manner…but,

The presence/absence of a target gene really tells us nothing about activity at the time of sampling

There are 5 approaches using RNA/DNA based methods to determine activity

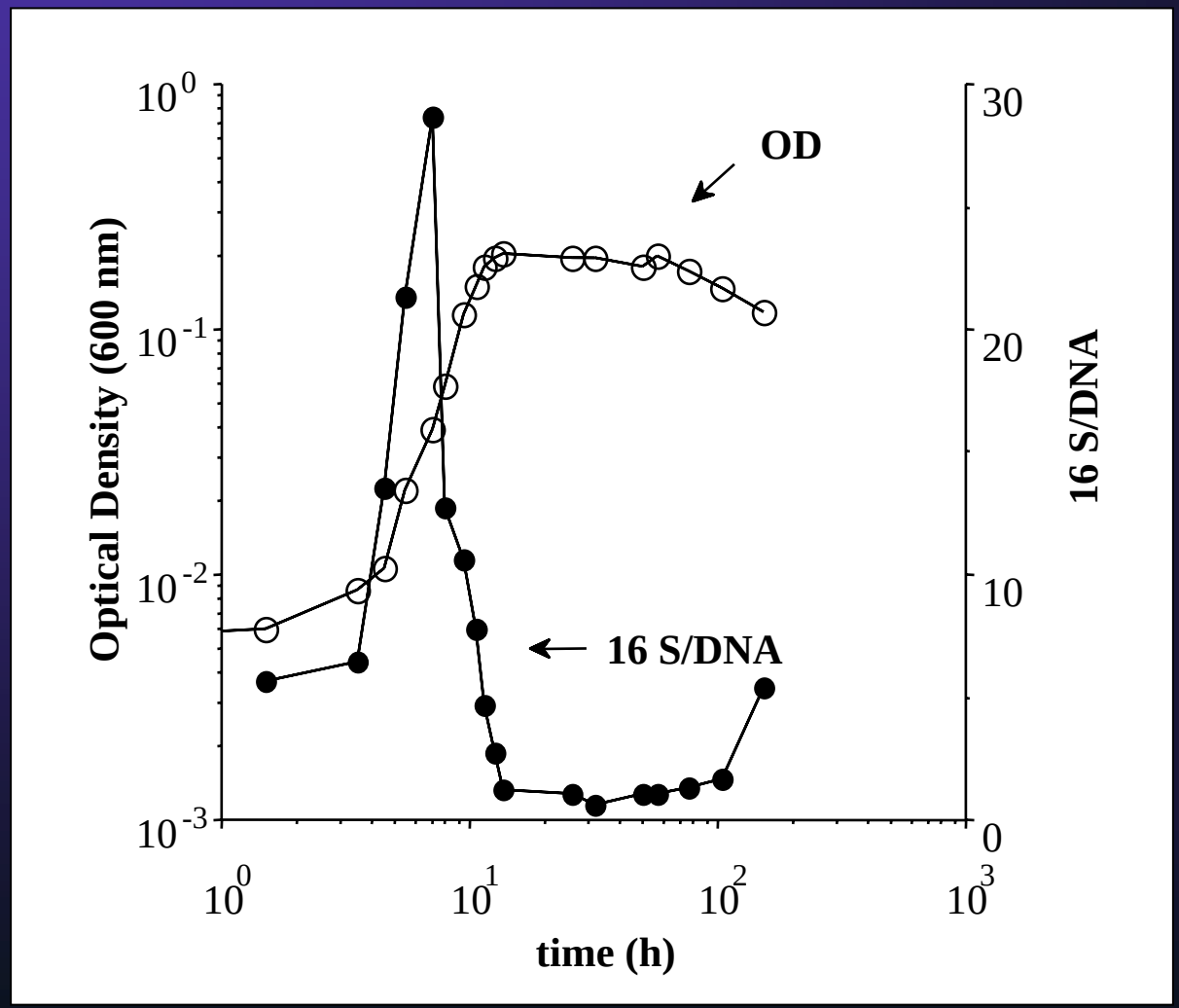
1. mRNA analysis
2. MICROFISH analysis
3. Ribosome fingerprinting
4. Stable Isotope incorporation
5. BrDU incorporation

Universal Ribosomal rRNA content vs. Growth Rate



Kerkhof and Ward, 1993

16 S rRNA/DNA Ratio of a Marine Bacterium During Batch Growth



Kerkhof and Kemp, 1999

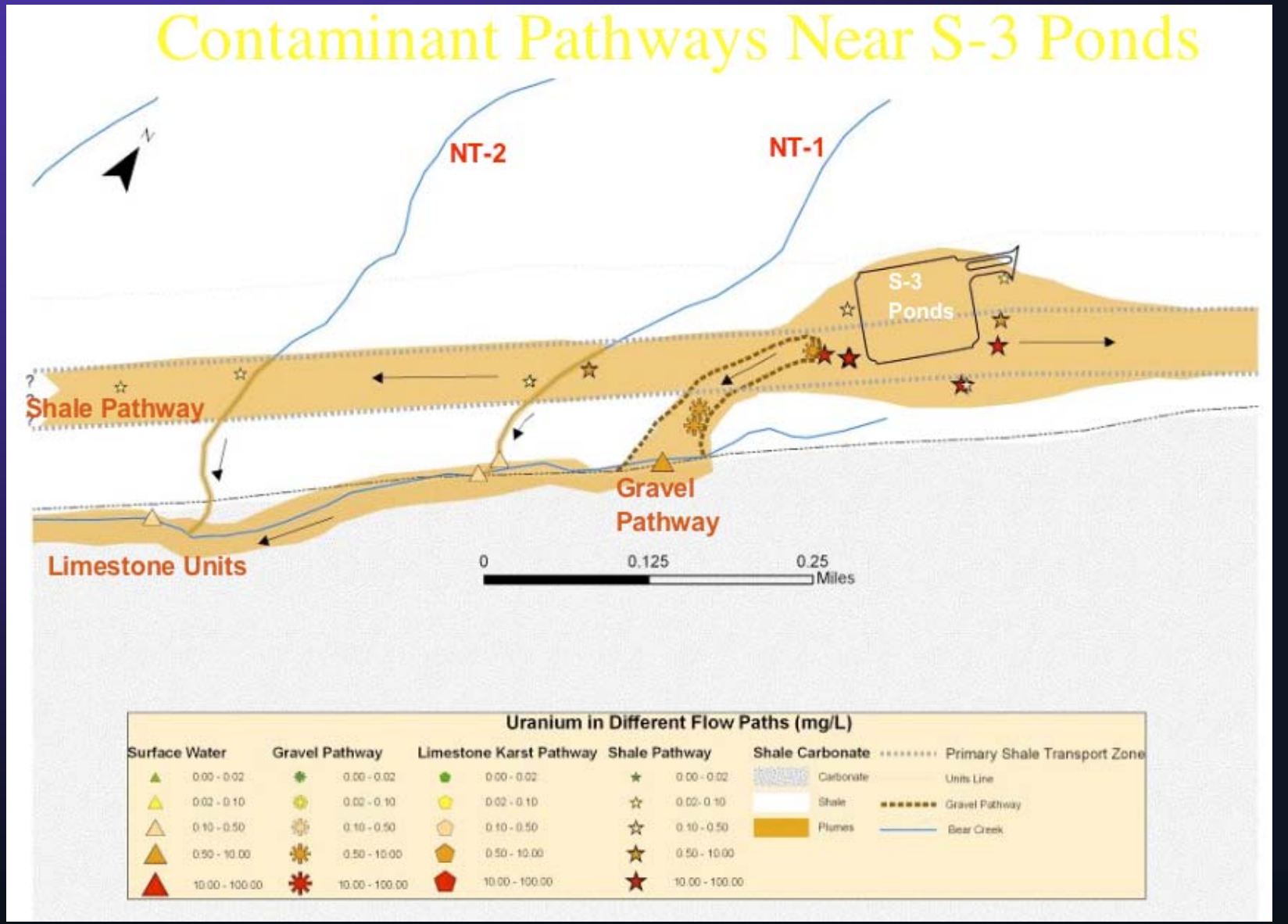
Experimental Approach

1) Obtain groundwater samples from a wide variety of wells at the FRC that correspond to a gradient of contaminants.

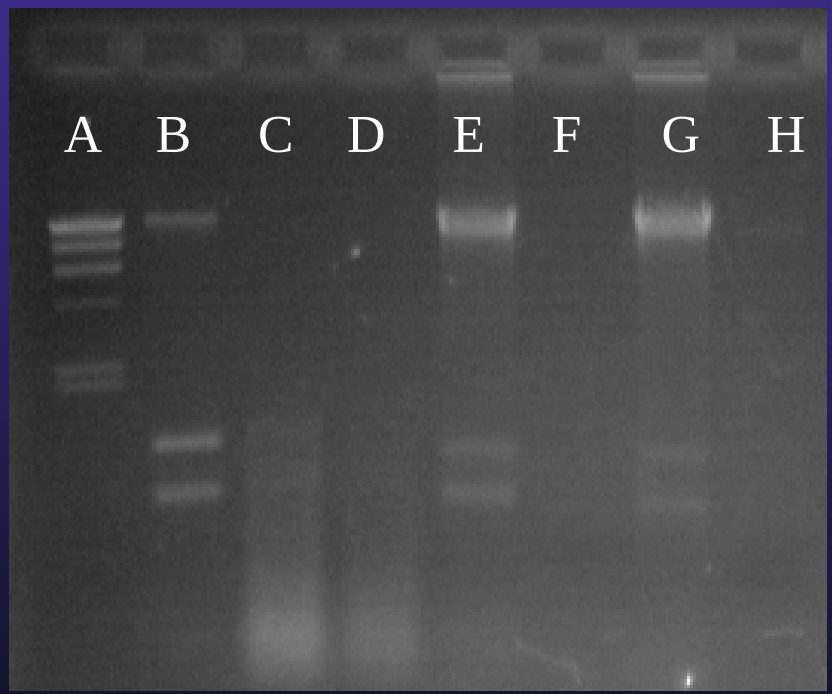
2) Fingerprint microbial populations at the DNA and ribosomal RNA level to assess temporal/spatial variability in presence/absence and activity

3) Verify rRNA activity measurements with incorporation of labels (e.g. bromodeoxyuridine or stable isotope)

Map of sample area at the FRC

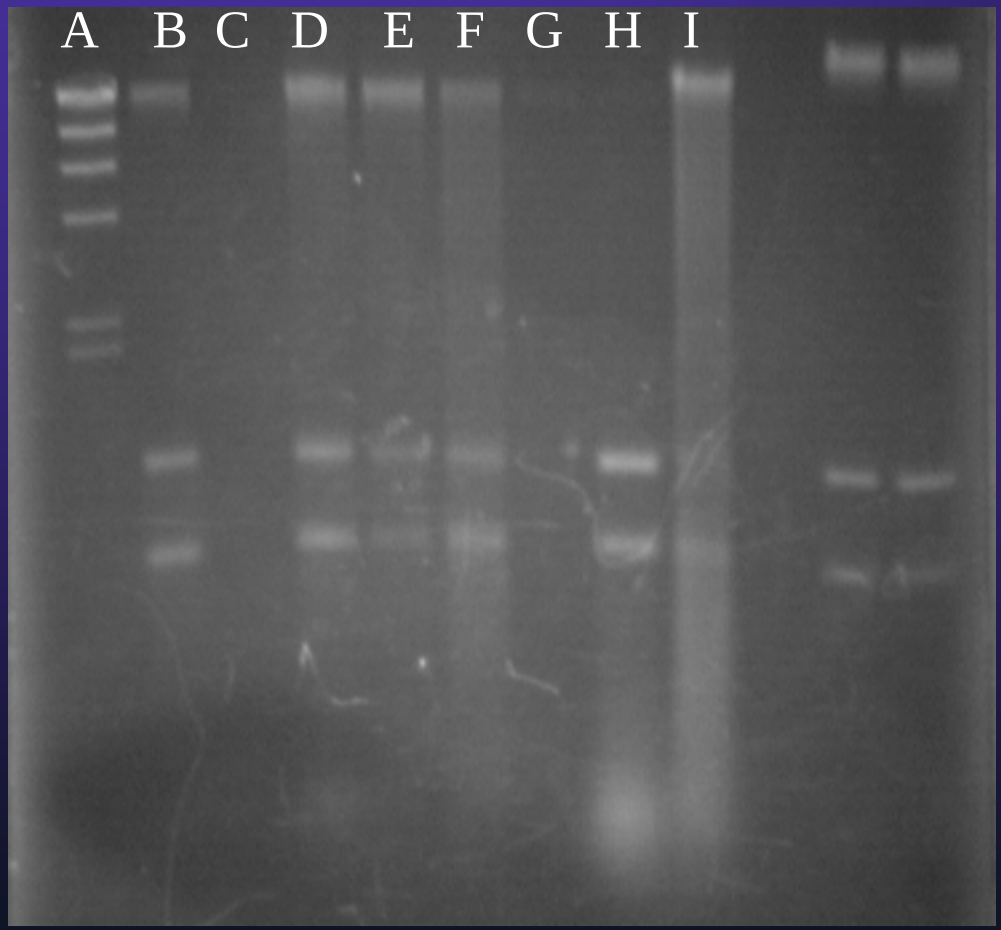


Agarose gel of extracts from Groundwater samples from FRC



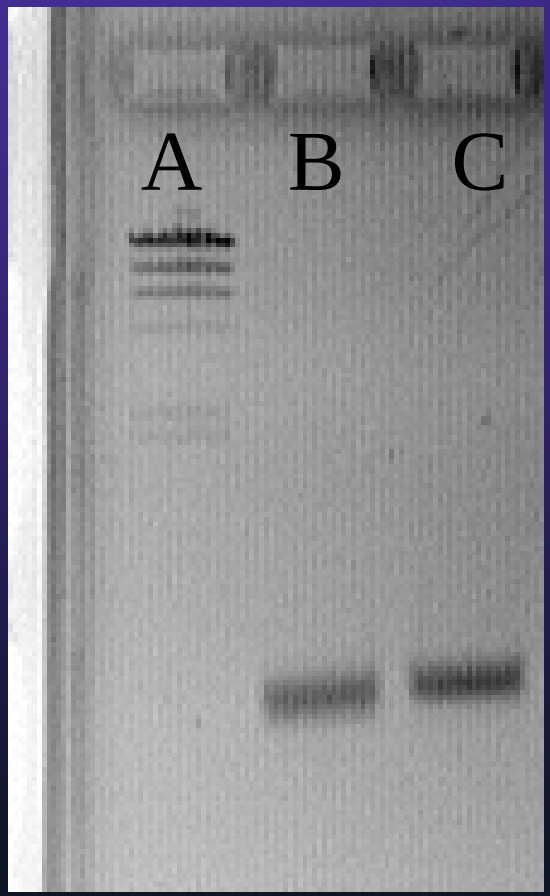
A) lambda std
B) DNA/RNA from culture
C) FW 106 stagnant (20m) ★
D) FW 106 low flow ★
E) TPB 16 (187m) ★
F) PTMW02 (340m)
G) GW346 (417m) ★
H) GW085 (917m)

Agarose gel of extracts from Groundwater samples from FRC



A) lambda std
B) DNA/RNA from culture
C) Blank
D) Bear Ck. 11.97
E) Spring S04
F) Bear Ck. 9.2
G) PTMW02
H) FW 413
I) Bear Ck. Seep

Agarose gel of RT-PCR reactions from Groundwater samples from FRC

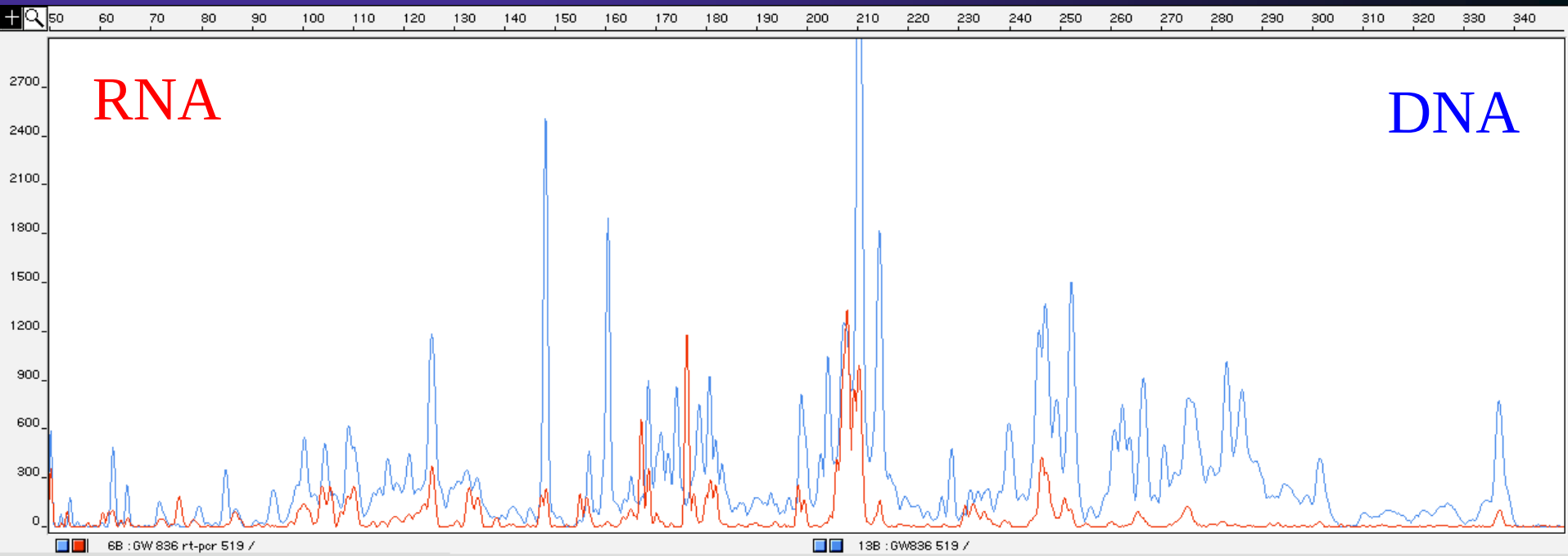


Sample A) lambda std
Sample B) GW 836 16S flo product
Sample C) Bottle incubation 16S flo product

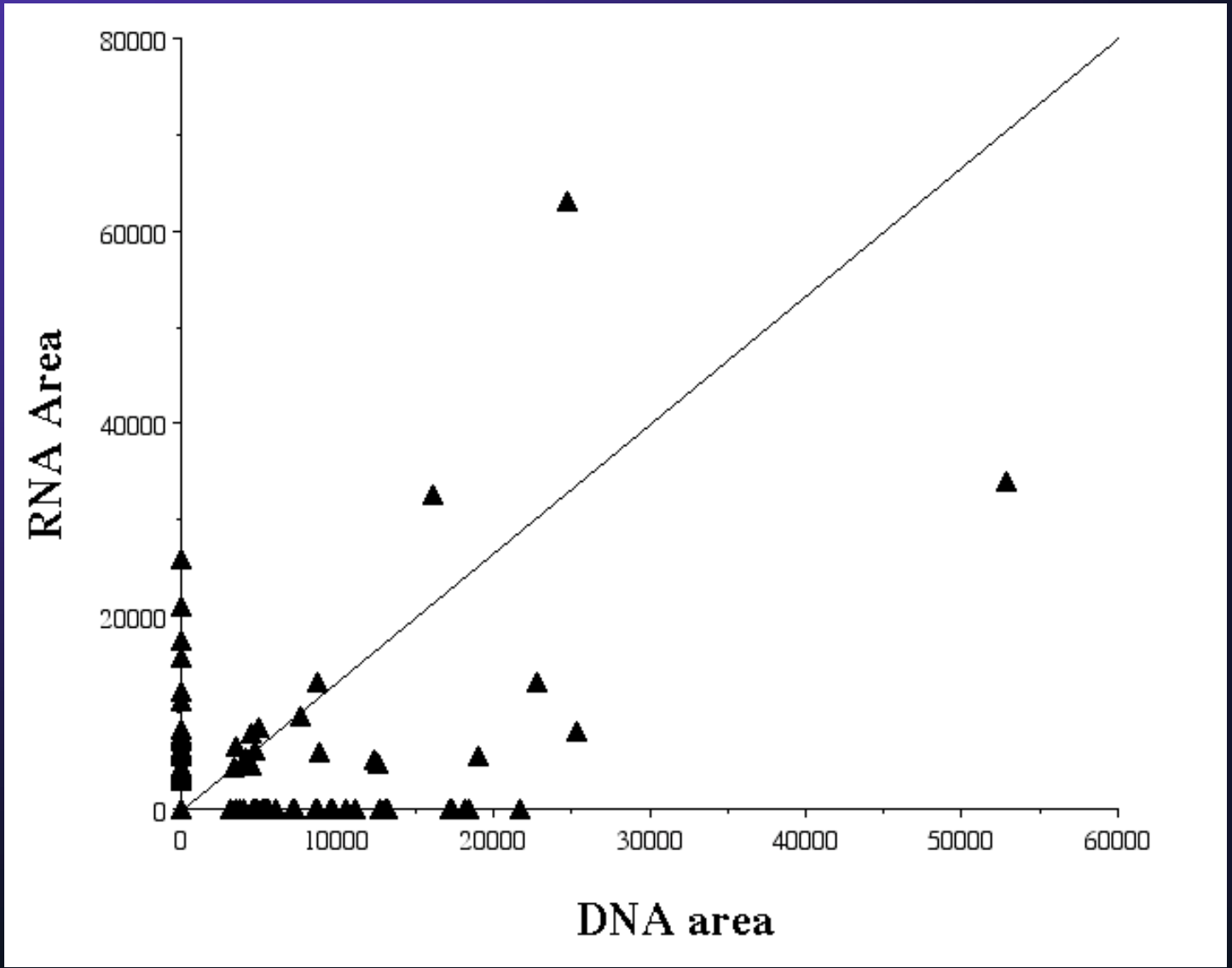
1:250 or 1:500 dilution of gel purified 16S rRNA subunit

* Also faint amp in 1:62,500 dilution of bottle incubated sample

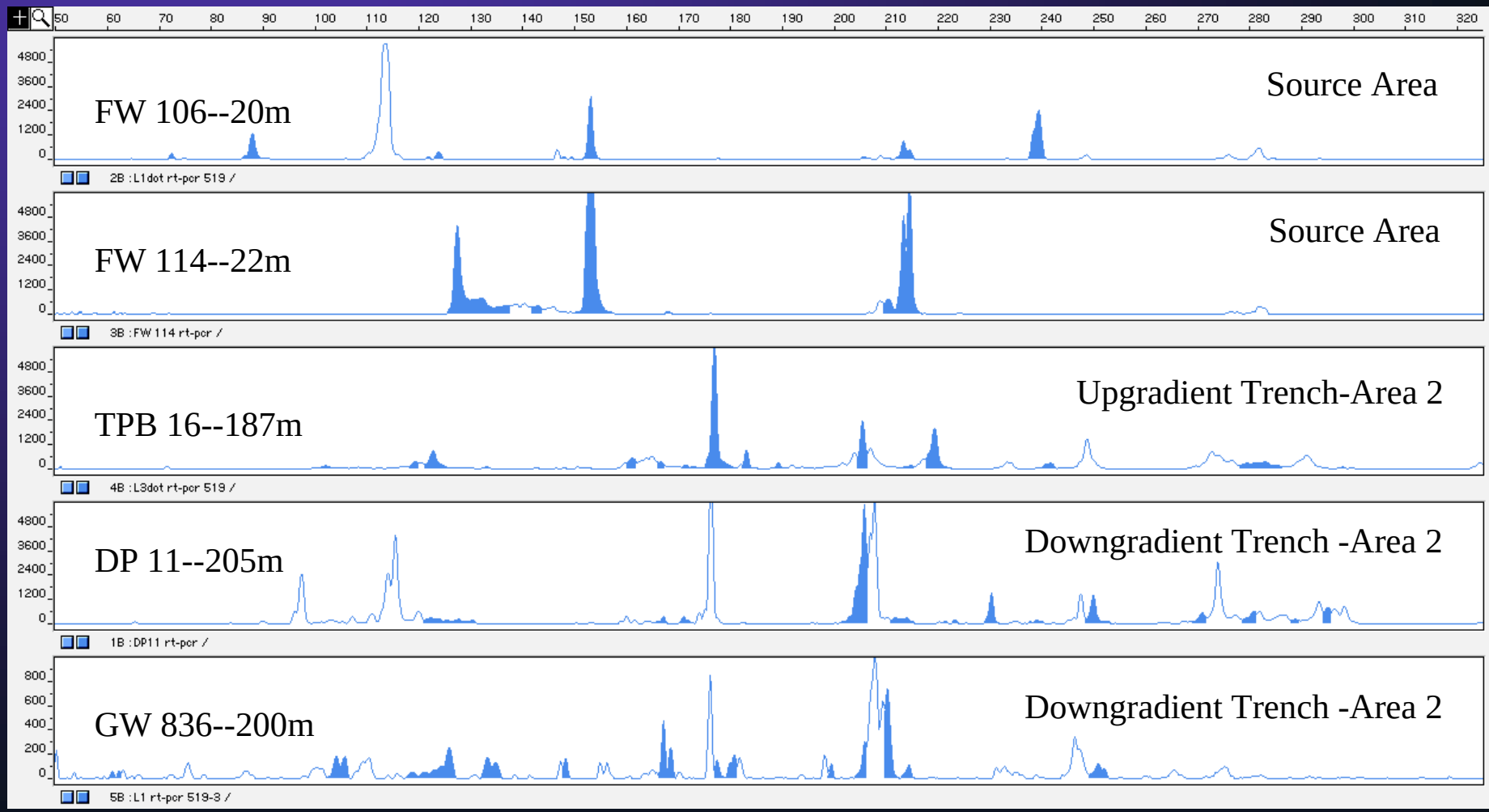
Comparison of DNA and RNA profiles for GW 836



Comparison of DNA and RNA peak areas from GW 836

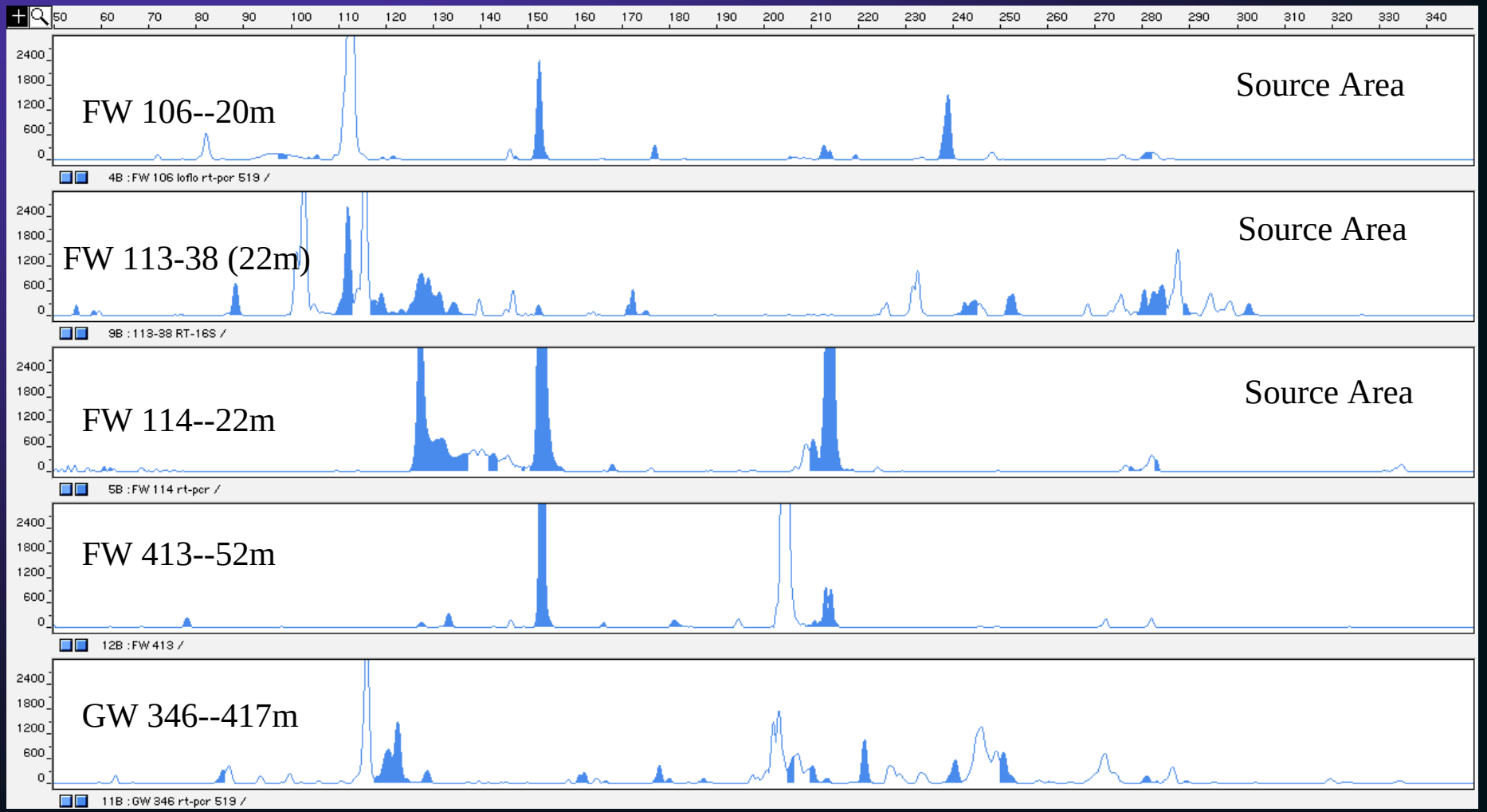


TRFLP profile of RT-PCR reactions along the Gravel Pathway at the FRC



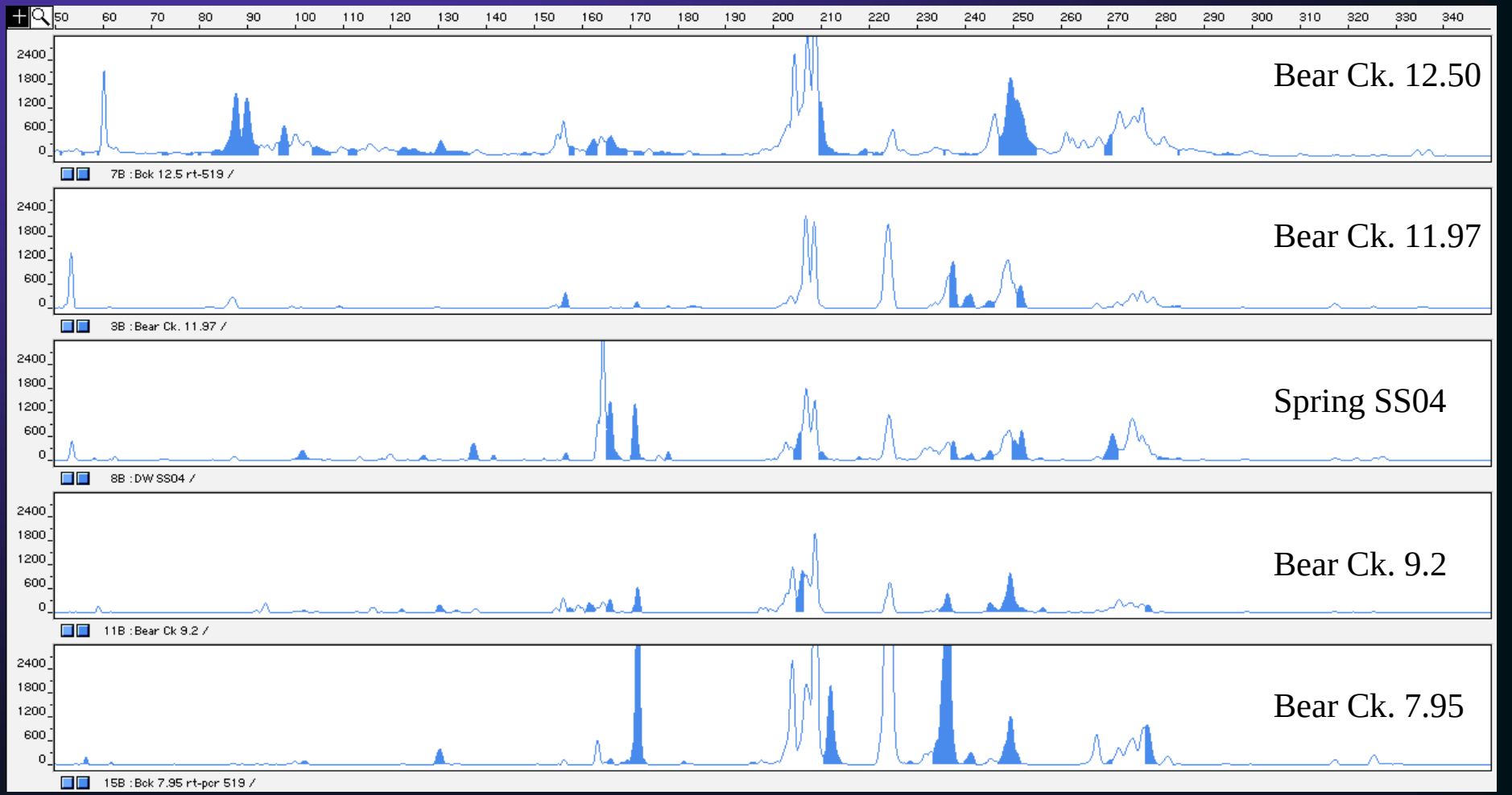
Clones from the FRC sequence database are highlighted in blue

TRFLP profile of RT-PCR reactions along the Shale Pathway at the FRC



Clones from the FRC sequence database are highlighted in blue

TRFLP profile of RT-PCR reactions along the Surface Water at the FRC

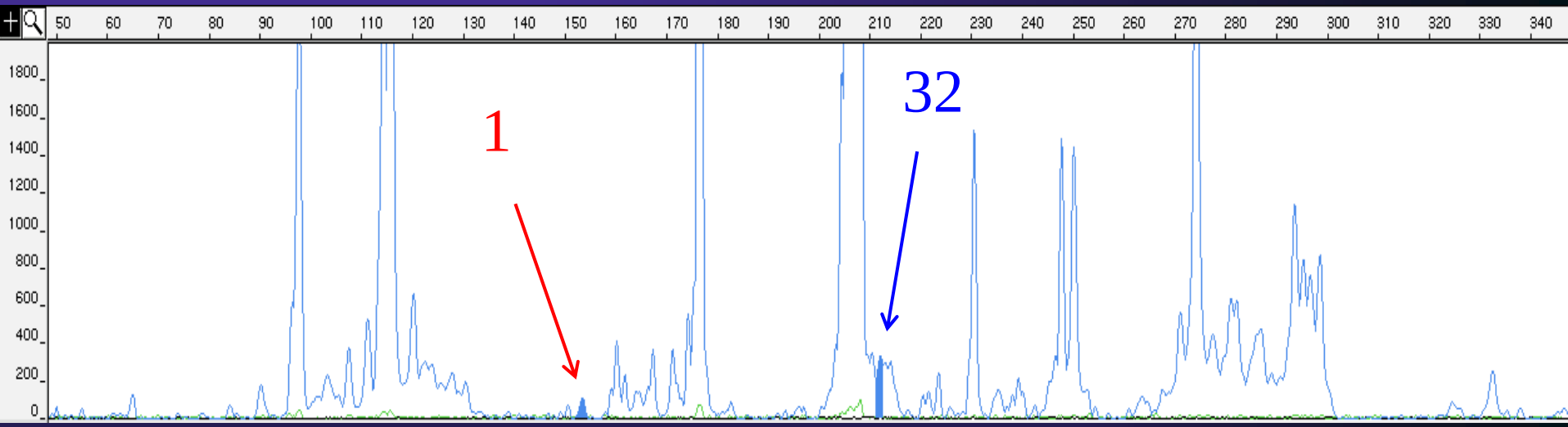


Coverage of TRFLP peaks by clonal libraries from FRC samples

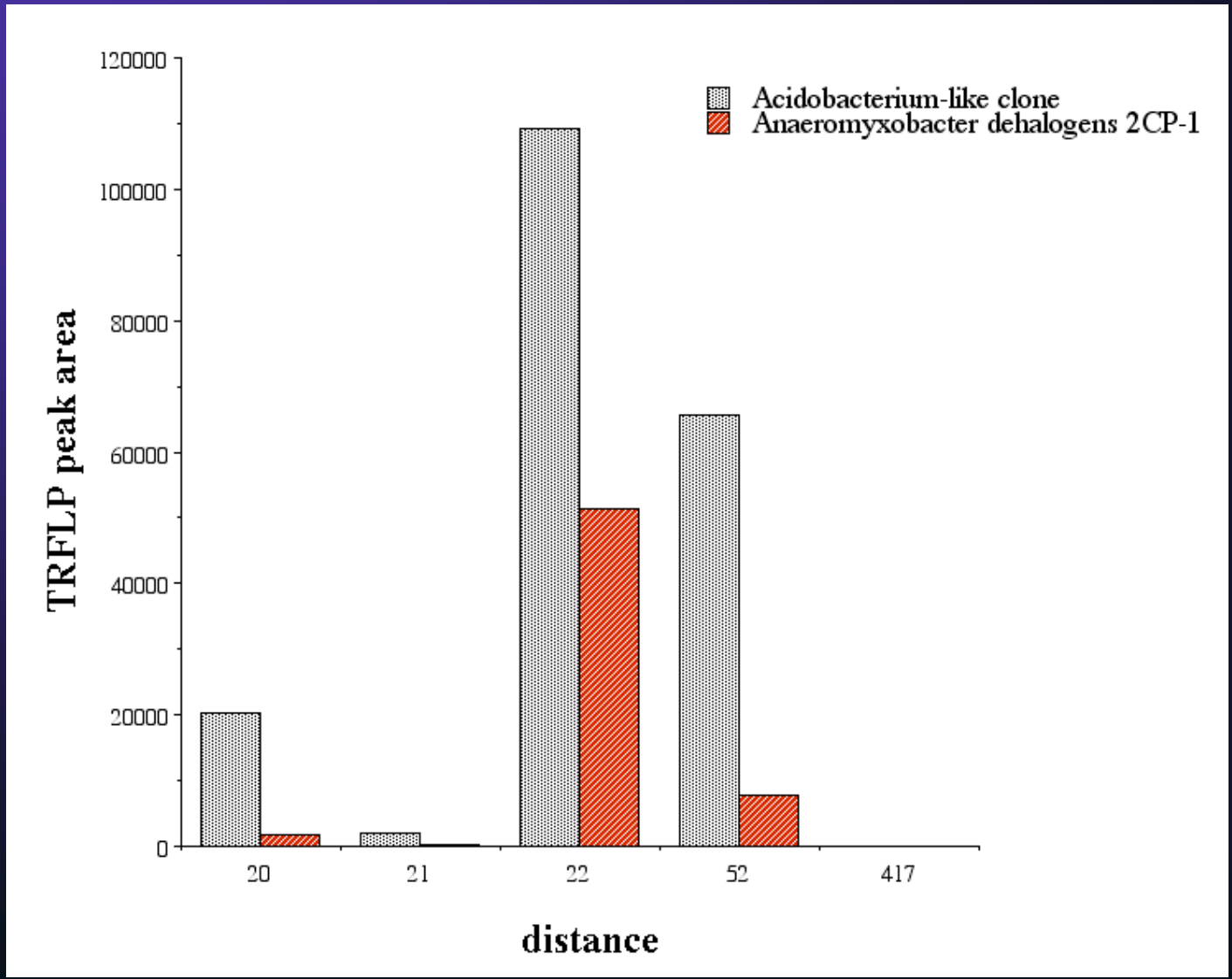
Shale pathway demonstrates 48 $\pm$ 7% coverage of the TRFLP peaks by 400 clones from Area 1, 2, and 3

Surface water pathway demonstrates 41 $\pm$ 4% coverage of the TRFLP peaks by 400 clones from Area 1, 2, and 3

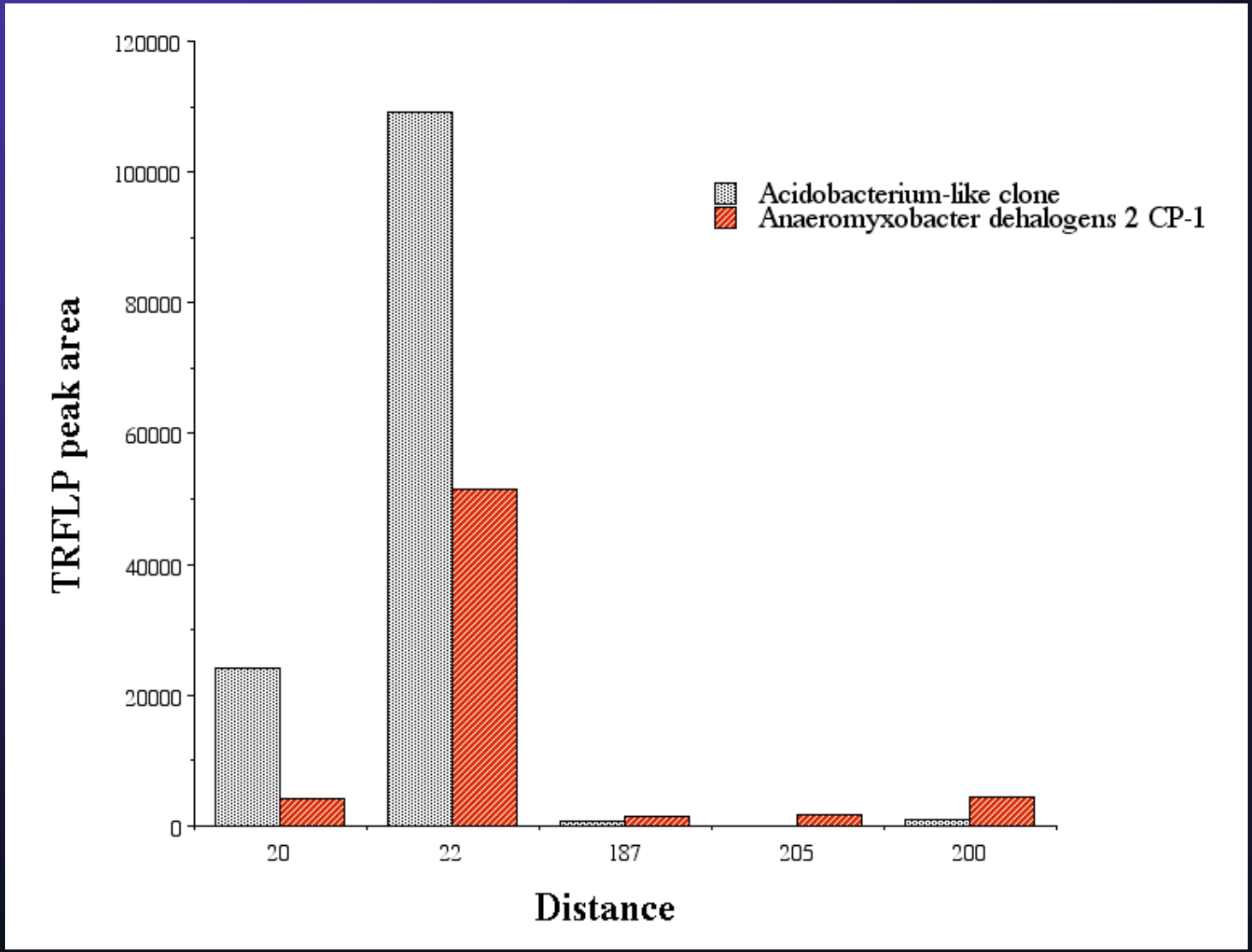
Comparison of clone frequency and TRFLP peak area for DP 11



TRFLP peak area from Shale Pathway

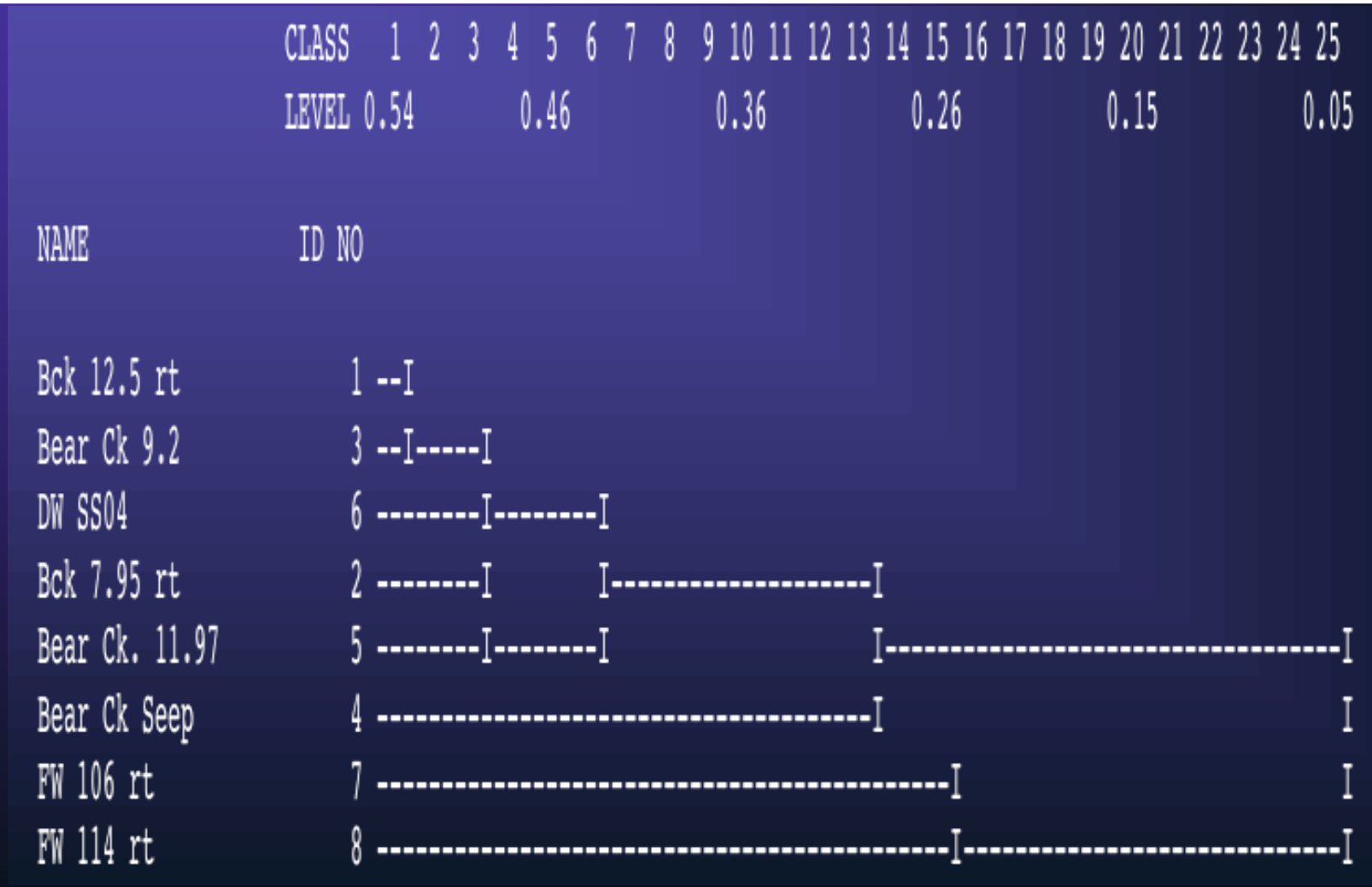


TRFLP peak area from Gravel Pathway



Genbank Search using sequence from the 153 bp peak

Cluster analysis of TRFLP peaks from Surface water samples



FRC Conclusions

- Extraction of intact ribosomes is reasonably easy from groundwater, but there is a need to modify the methods for some shale pathway samples.
- Fingerprinting of ribosomes is nearly complete . Different populations are active in different areas of the contaminant plume. More diversity w/less contamination
- There is little correspondence between the DNA peak areas and the RNA peak areas at GW-836.
- Changes in the active fraction of the bacterial community can be observed downgradient.
- Many of the major peaks observed in the RT-PCR fingerprints have been cloned (roughly 50%). However, many major peaks remain to be discovered.

Overall Conclusions for Omics

- The “Omics” have great potential to tell us a tremendous amount of information regarding bacteria in the environment.
- I’d urge that we also consider using the tools/methods currently at use in our labs for high throughput screening of environmental samples to target the abundant and active organisms
- It helps to know what you are looking for.

Where in the PCR cycle was all the bias research done?

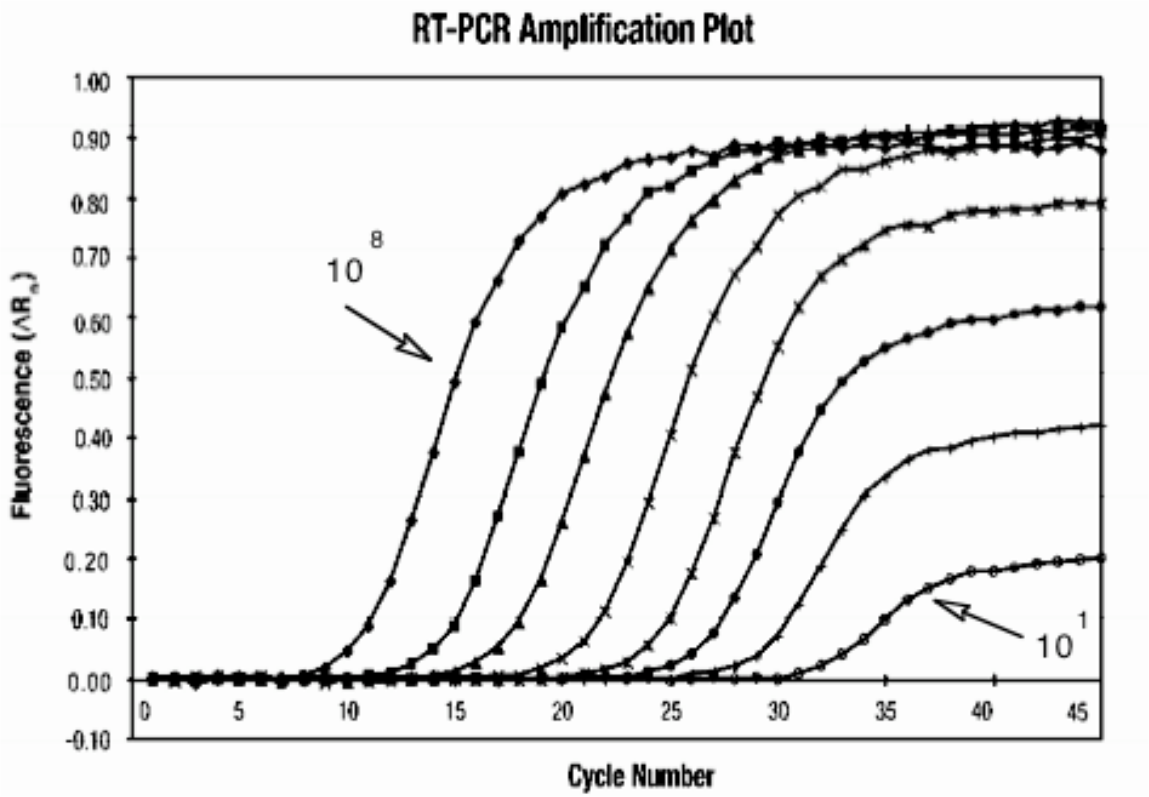


Figure 3. Quantitation of RT- PCR product. Data from Perkin-Elmer (http://www2.perkin-elmer.com:80/ab/about/pcr/sds/wpfig2.html)

Variation of replicate samples from 5 bioreactors

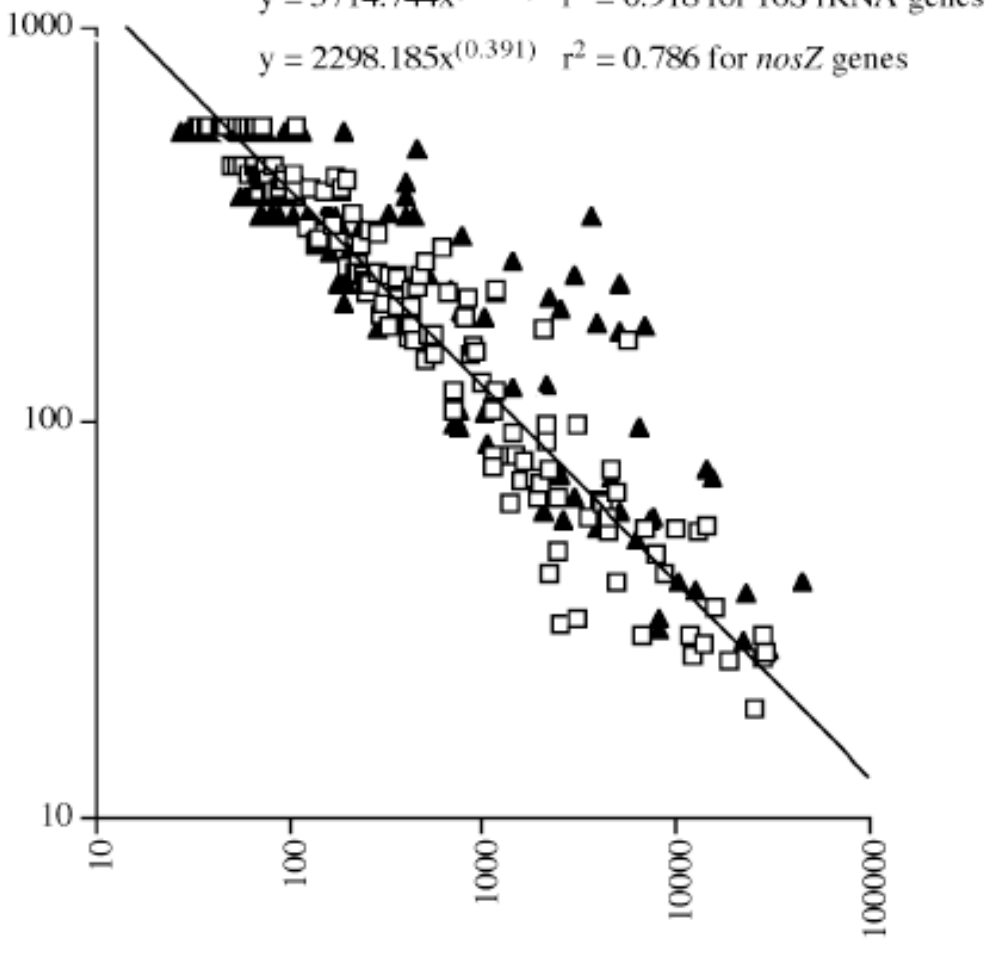
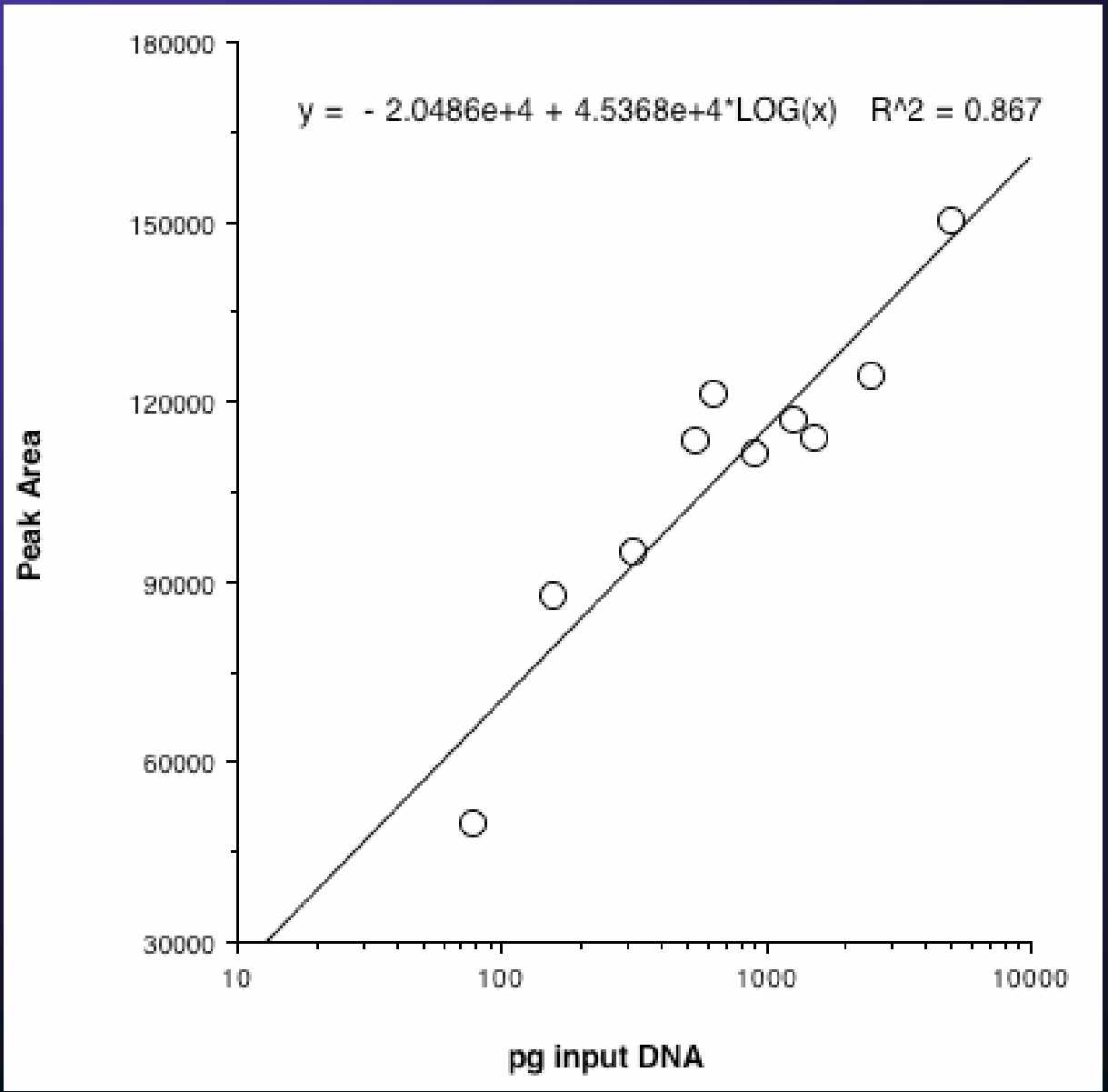
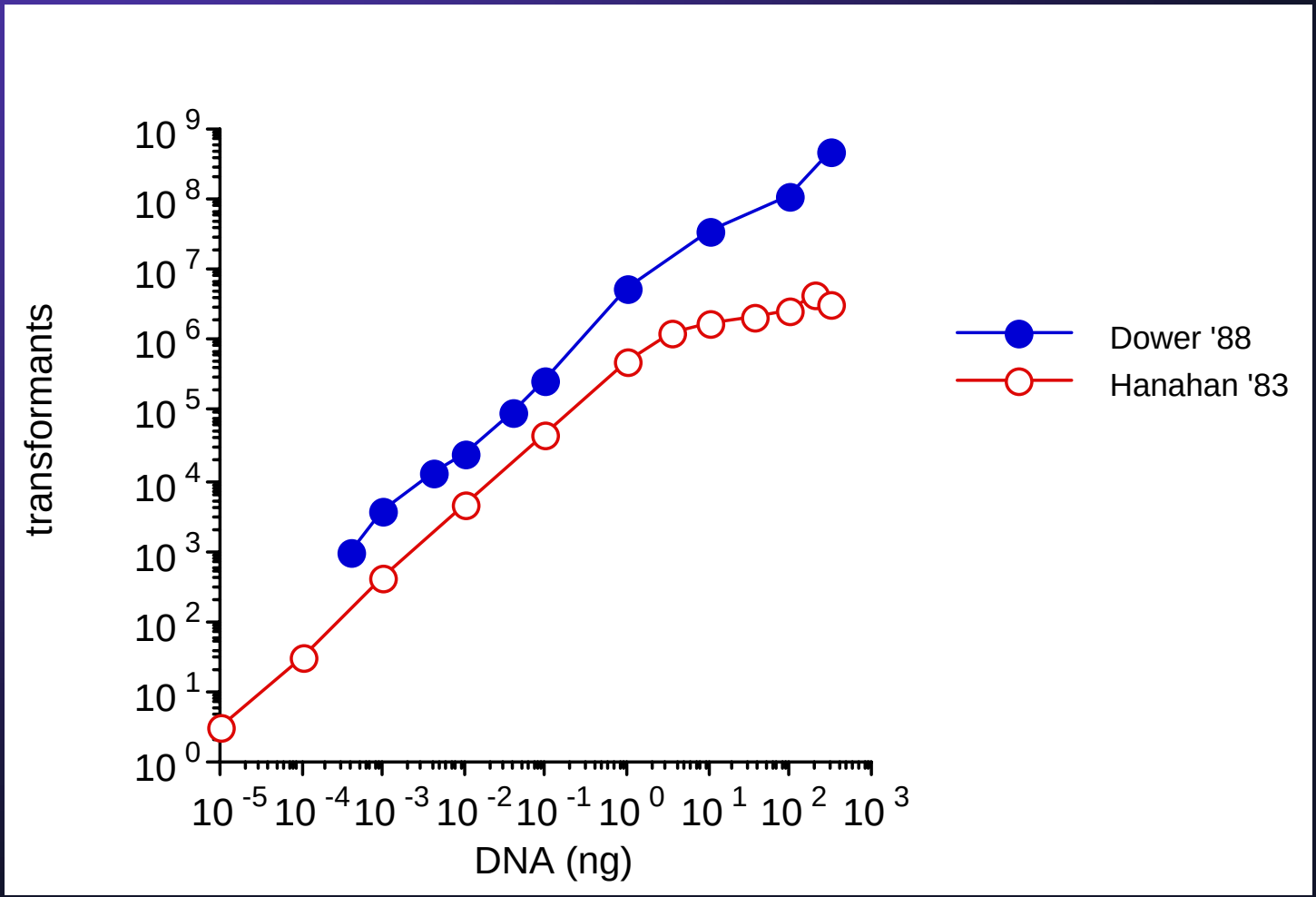


FIGURE 8. Mean peak area vs coefficient of variation for 16srRNA (□) and *nosZ* (▲) for all samples.

Do bigger peaks represent more target gene?



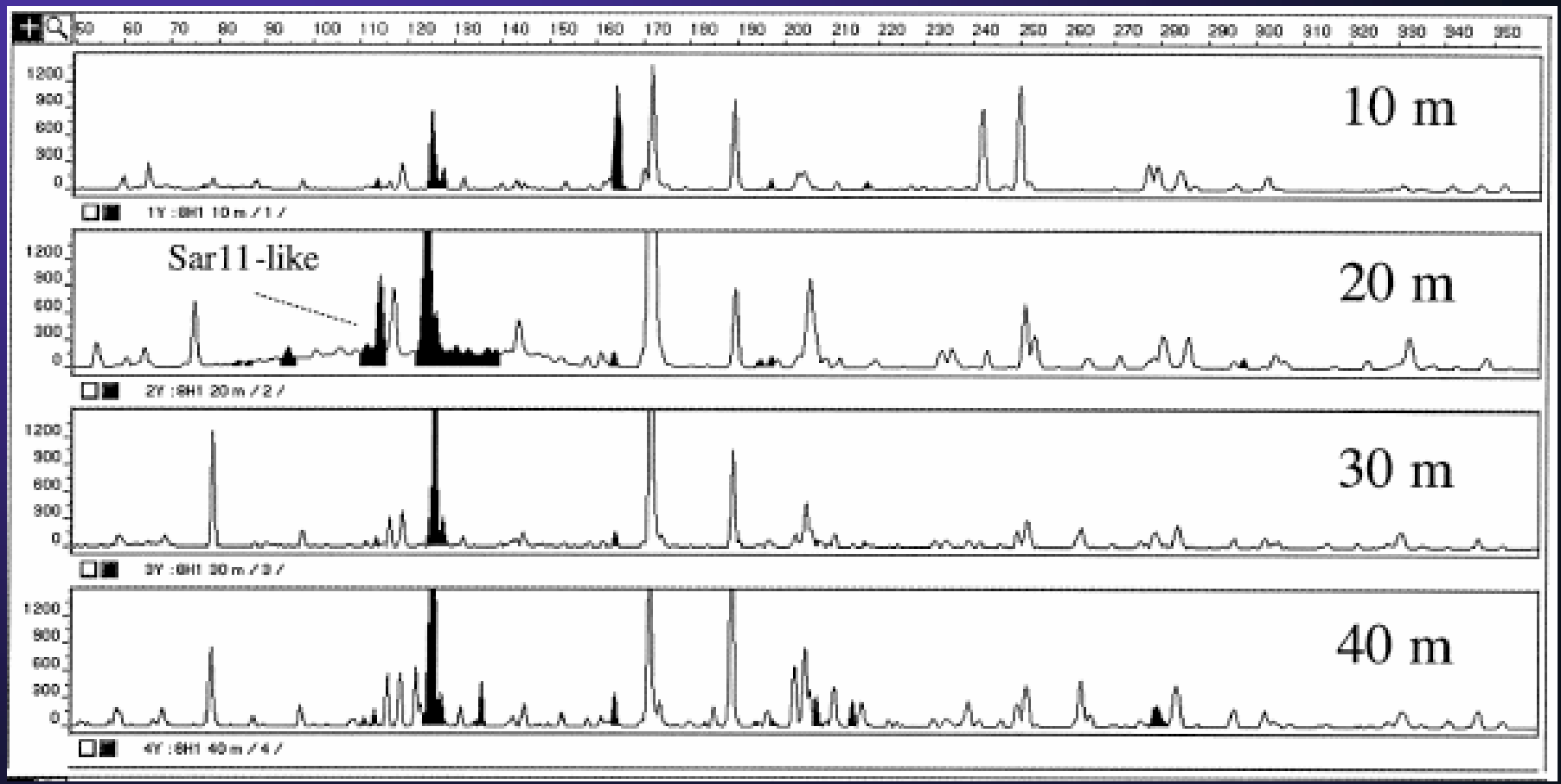
Why should we expect to have lots of clones from big TRFLP peaks or DGGE bands?



Dower/Hannahan: Relationship between total DNA mass and number of transformants.

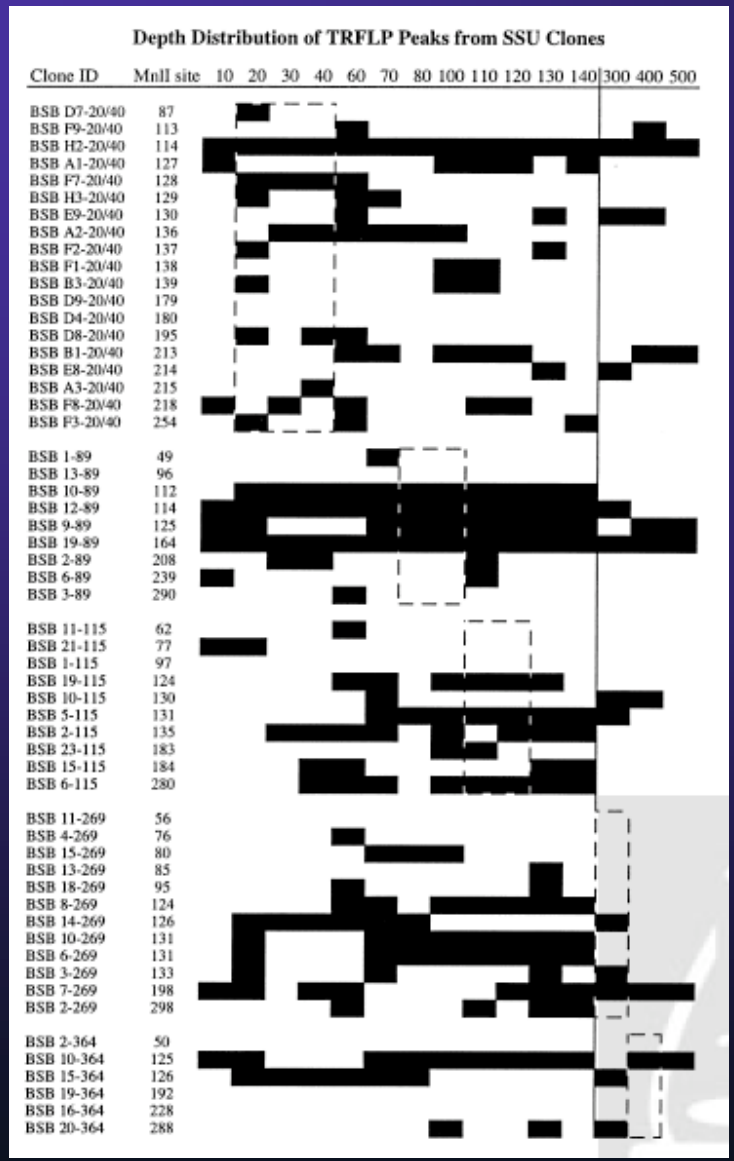
Evidence in Literature for Cloning Bias

Vetriani *et al* 2003



TRFLP community profiles of microbial consortia at different depths. Highlighted peaks are the only peaks that could be isolated from the clone library.

Evidence in Literature for Cloning Bias



Vetriani *et al* 2003